

SEQUENCE LISTINGS

<110> Hanmi Pharm. Co., Ltd.

<120> EXPRESSION VECTOR FOR SECRETING ANTIBODY FRAGMENT USING E. COLI SIGNAL
SEQUENCE AND METHOD FOR MASS-PRODUCING ANTIBODY FRAGMENT

<130> Q94300

<140> 10/576,068

<141> 2006-04-14

<150> KR1020030072216

<151> 2003-10-16

<150> PCT/KR04/02625

<151> 2004-10-14

<160> 36

<170> KopatentIn 1.71

<210> 1

<211> 75

<212> DNA

<213> Artificial Sequence

<220>

<223> gene fragment of light chain variable region

<400> 1
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ggggacagag tcacc 75

<210> 2

<211> 80

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<223> gene fragment of light chain variable region

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tgggtttttgc tgataccagg ctaagtaatt tctgatgccc tgacttgccc gacaagtgat 60
ggtgactctg tccctacag 80

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<211> 80

<212> DNA

<213> Artificial Sequence

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 tgcaatcagg ggtcccatct 80

<210> 4
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<400> 4
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 agatgggacc cctgattgca 80

<210> 5
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 caccgtatac ttttgccag 80

<210> 6
 <211> 41
 <212> DNA
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<400> 6
 tttgatttcc accttggtcc cctggccaaa agtatacggt g 41

<210> 7
 <211> 75
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> gene fragment of heavy chain variable region

<400> 7
 gggaagcttc gatcggaggt gcagctgggtg gagtctgggg gaggtctggt acagcccgcc 60
 aggtccctga gactc 75

<210> 8
 <211> 79
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> gene fragment of heavy chain variable region

<400> 8
 agcttgccgg acccagtgc tggcataatc atcaaagggt aatccagagg ccgcacagga 60
 gagtctcagg gacctgccg 79

<210> 9
 <211> 80
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<220>
 <223> gene fragment of heavy chain variable region

<400> 9
 tgcactgggt ccggcaagct ccagggaagg gcttggaatg ggtctcagct atcacttgga 60
 atagtgtgca catagactat 80

<210> 10
 <211> 80
 <212> DNA
 <213> Artificial Sequence

<220>
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<400> 10
 atacaggggag ttcttggcgt tgtctctgga gatgggtgaat cggccctcca cagagtccgc 60
 atagtctatg tgaccactat 80

<210> 11
 <211> 80
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> gene fragment of heavy chain variable region

<400> 11
 acgccaagaa ctccctgtat ctgcaaatga acagtctgag agctgaggat acggccgtat 60
 attactgtgc gaaagtctcg 80

<210> 12
 <211> 84
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> gene fragment of heavy chain variable region

<400> 12
 cactcgagac ggtgaccagg gtacctggc cccaatagtc aaggaggagac gcggtgctaa 60
 ggtacgagac ttctgcacag taat 84

<210> 13
 <211> 39
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> RT-PCR forward primer specific for heavy chain

<400> 13
 cccaagctta ggctccacc aaggggcccat cggctcttcc 39

<210> 14
 <211> 48
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> RT-PCR reverse primer specific for heavy chain

<400> 14
 gggggatcct tatgggcacg gtgggcattgt gtgagttttg tcacaaga 48

<210> 15
 <211> 42
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> RT-PCR forward primer specific for light chain

<400> 15

cccaagcttt cgcaactgt ggctgcacca tctgtttca tc 42

<210> 16
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> RT-PCR reverse primer specific for light chain

<400> 16
ccgggatccc taacactctc ccctgttgaa gctctttgtg ac 42

<210> 17
<211> 69
<212> DNA
<213> modified E. coli thermostable enterotoxin II signal sequence

<400> 17
atgaaaaaga caatgcgatt tcttcttgca tctatgttcg ttttttctat tgctacaaat 60
gcccaggcg 69

<210> 18
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> forward primer containing StuI restriction enzyme site

<400> 18
tctattgcta caaatgccca ggccttccca accattccct tatcc 45

<210> 19
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> reverse primer containing StuI restriction enzyme site

<400> 19
agataacgat gtttacgggt ccggaagggt tggttaaggga atagg 45

<210> 20
<211> 51
<212> DNA
<213> Artificial Sequence

<220>

<223> reverse primer specific for light chain

<400> 20
gggggatcct cagcgggcg atgtgtgagt ttgtcacaa gatttaggct c 51

<210> 21
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> forward primer containing SD sequence and BamHI restriction enzyme site

<400> 21
gggggatcca ggaggtgatt tatgaaaaa acaatcgcat ttc 43

<210> 22
<211> 44
<212> DNA
<213> Artificial Sequence

<220>
<223> forward primer containing BpuI restriction enzyme site

<400> 22
ggggctgagc aggaggtgat ttatgaaaaa gacaatcgca ttcc 44

<210> 23
<211> 52
<212> DNA
<213> Artificial Sequence

<220>
<223> reverse primer containing BpuI restriction enzyme site

<400> 23
ggggctcagc tcacggggcg catgtgtgag tttgtcaca agatttaggc tc 52

<210> 24
<211> 63
<212> DNA
<213> E. coli OmpA signal sequence

<400> 24
atgaaaaaga cagctatcgc gattgcagtg gcactggctg gtttcgctac cgttgcgcaa 60
gct 63

<210> 25
<211> 30

<212> DNA
 <213> Artificial Sequence

 <220>
 <223> forward primer specific for heavy chain

 <400> 25
 gaggttcagc tagtcgagtc aggaggcggt 30

 <210> 26
 <211> 51
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> forward primer containing HindIII and StuI restriction enzyme sites

 <400> 26
 gggagatctt cacgcggcgc atgtgtgagt ttgtcaca gatttaggt c 51

 <210> 27
 <211> 30
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> reverse primer containing stop codon and BamHI restriction enzyme site

 <400> 27
 gacattcaaa tgacccagag cccatccagc 30

 <210> 28
 <211> 42
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> forward primer containing HindIII and NruI restriction enzyme sites

 <400> 28
 cccagatctc taacactctc cctcttgaa gctctttgtg ac 42

 <210> 29
 <211> 41
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> reverse primer containing stop codon and BamHI restriction enzyme site

<400> 29
 ggggtcgaca ggaggtgatt tatgaaaaag acagctatcg c 41

<210> 30
 <211> 51
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> reverse primer containing SalI restriction enzyme site

<400> 30
 ggggtcgact caccggcgcc atgtgtgagt ttgtcaca gatttaggct c 51

<210> 31
 <211> 42
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> forward primer specific for modified E. coli enterotoxin II signal peptide and containing NdeI restriction enzyme site

<400> 31
 gggcatatga aaaagacaat cgcatttctt ctgcatceta tg 42

<210> 32
 <211> 705
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> TNF-alpha heavy chain

<400> 32
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 tcctgtgcgg cctctggatt cactcttgat gattatgcca tgcactgggt ccggcaagct 120
 ccagggaagg gcttgaatg ggtctcagct atcacttgga atagtggcca catagactat 180
 gcggactctg tggagggcgg attcaccate tcagagaca acgccaagaa ctccctgtat 240
 ctgcaaatga acagtctgag agctgaggat acggccgtat attactgtgc gaaagtctcg 300
 taccttagca ccgcgtcttc ccttgactat tggggccaag gtaccttggt caccgtctcg 360
 agtgccctca ccaaggggcc atcgggtcttc ccctggcac cctctccaa gagcacctct 420
 gggggcacag cgccctggg ctgcctggtc aaggactact tcccgaacc ggtgacggtg 480
 tcgtggaact caggcgccct gaccagcggc gtgcacacct tcccggtgtg cctacagtcc 540

tcaggactct actcectcag cagcgtggtg accgtgcect ccagcagctt gggcaccacg	600
acctacatct gcaacgtgaa tcacaagccc agcaacacca aggtggacaa gaaagttgag	660
cccaaattct gtgacaaaac tcacacatgc ccaccgtgcc catag	705

<210> 33
 <211> 645
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> TNF-alpha light chain

<400> 33	
gacatccaga tgaccagtc tccatcctcc ctgtctgcat ctgtaggagg cagagtcacc	60
atcacttgct gggcaagtca gggcatcaga aattacttag cctggtatca gcaaaaaacca	120
gggaaagccc ctaagctcct gatctatgct gcattcaact tgcaatcagg ggtcccatct	180
cggttcagtg cagctggatc tgggacagat ttcaactcga ccattcagag cctacagcct	240
gaagatgttg caacttatta ctgtcaaaag tataaccgtg caccgtatac ttttggccag	300
gggaccaagg tggaaatcaa acgaactgtg gctgcacat ctgtcttcat cttcccgcca	360
tctgatgagc agttgaaate tggaactgcc tctgttgtgt gcctgctgaa taactcttat	420
cccagagagg ccaaagtaca gtggaaggtg gataacgccc tccaatcggg taactcccag	480
gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg	540
ctgagcaaaag cagactacga gaaacacaaa gtctacgctc gcgaagtcac ccattcagggc	600
ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gttag	645

<210> 34
 <211> 7
 <212> PRT
 <213> TNF-alpha light chain

<400> 34
 Asp Ile Gln Met Thr Gln Ser
 1 5

<210> 35
 <211> 8
 <212> PRT
 <213> TNF-alpha heavy chain

<400> 35
 Glu Val Gln Leu Glu Val Asp Ser
 1 5

<210> 36
 <211> 12
 <212> PRT
 <213> N-terminal sequence of recombinant TNF-alpha

<400> 36
 Asp Glu Ile Val Gln Met Leu Thr Val Gln Asp Ser
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